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False smut of rice (*Ustilaginoidea virens*) under temperate agro-climatic conditions of Kashmir, India

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ABSTRACT
False smut, caused by *Ustilaginoidea virens*, has recently been found in temperate agro-climatic conditions of Kashmir and is sporadic where rice is cultivated. In this study, rice false smut was surveyed in major rice production areas of Kashmir during two seasons. Disease incidence and severity varied significantly between seasons and locations. Seed germination in smut infected samples was 72.4% which is 21.45 per cent lower than the non - smutted samples (92.20%). Shoot and rood length were reduced to the extent of 32.53 and 22.65 per cent, respectively. Rice genotypes Jhelum and Shalimar rice-1 were found resistant while Pusa sughandh 3 and 5 were highly susceptible to false smut. Kashmir valley being mono cropped area of rice in Kharif season, further studies are needed to investigate the viability of the fungal spores and sclerotia during winter, variation between fungal isolates, ability to produce mycotoxins and devise control measures using integrated disease management practices.

Introduction

Rice (*Oryza sativa* L.) is an important cereal crop and staple food of Kashmir. Its demand is increasing day by day with the increase in human population. It is grown in both temperate and sub temperate zones of valley within an altitude of 1650m to 2200m amsl (above mean sea level) representing temperate region of the country (Ahmad et al. 1999). The rice crop in valley is frequently challenged by various biotic and abiotic stresses throughout the growing season. Though during past two and half decades, a few high yielding rice varieties have been released, rice productivity in the valley has reached a plateau in the recent years and chances of further yield enhancement are scanty due to low genetic variability in hill rice cultivars (Sanghera and Wani 2008). The diversity in agro-climate, coupled with farmers' preferences, give rise to wide range of grain preference from bold and coarse to fine grains in temperate regions. In Kashmir valley, rice occupies an area of 0.141 million ha with annual production of 0.34 million tons and average productivity 2.5t/ha (Anonymous 2009).

In Kashmir, breeding programs in this crop has reached to a point of diminishing returns and it is feared that unless new diversity is infused into the breeding germplasm, we face catastrophic reductions in productivity if the climate turns adverse (Sanghera and Rather 2011). Armed with the understanding of specific adaptation, the local constraints to production in the context of climate change and the changing consumer requirements of different geographic areas for new cultivars. It is worthwhile to highlight that hybridization between diverse germplasm followed by selection may result in cultivars with improved stress tolerance, better adaptation, quality traits and yield, besides the crop is exposed to a wide range of disease pressure.

In the present climate change scenario rice crop is facing the tough competition of new diseases which were otherwise not touching the economical threshold. Smut (*Ustilaginoidea virens*) disease is emerging as one of the potential threats to rice cultivation under Kashmir agroclimatic conditions. The incidence of this disease is becoming a major constraint to adoption of modern rice cultivars in temperate conditions, which occurs sporadically every year but may occur in severe form (endemic form) in such a growing season when, water stress, medium temperature (25-30^{0}C), cloudy weather with high humidity (>90%) prevails for a longer period result in tremendous losses in rice production (Bhagat and Prasad 1996; Yashoda et al. 2000). Late sowing and application of high Nitrogen doses favours the development of disease (Ahonsi et al. 2000; Li et al. 1986). This has also been reported from other rice growing countries of the world and has emerged in recent years as one of the most devastating grain disease (Zhou et al. 2008). The yield loss estimates ranged from 0.2 to 49 per cent in different regions with different rice varieties (Biswas 2001; Singh et al. 1992; Li et al. 1986). The disease affects the grains and the symptoms produced are visible only after flowering. *U. virens* infects the young ovary of individual spikelets and converts them into large velvety green smut balls. Infected rice during the flowering stage inhibited flower fertility and development of adjacent spikelets. The lower part of spikes is generally more severely infected than upper part. Smut balls are initially yellow in colour and are covered by a membrane, later the membrane bursts and the colour changes to yellowish green and finally greenish black. The international rice breeding programmes at the International Rice Research Institute (IRRI), and Indian research institutions under ICAR umbrella aim to develop cultivars/ genetic stocks adapted to changing climates in diverse agro-ecologies and farmers' preference. Therefore, the present work reports the status of rice false smut disease under temperate agroclimatic conditions of Kashmir and the reaction of some fine grained rice genotypes to false smut. In view of the

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importance of false smut disease of rice and the damage caused, there is urgent need to develop suitable/appropriate disease management strategies.

Materials and methods

False smut is a new disease in Kashmir, it occurred for the first time in the Budgam District in 2008 on fine grained aromatic rice variety Pusa Sugandh 3 and since then it appeared annually. The infected grains were transformed into large, velvety, green masses, which exceeded more than twice the diameter of the normal grain. The chlamydospores in the initial stages of development are orange yellow, then they turn dark green or almost black. The typical symptoms of this disease had been described by Verma and Singh (1988). In present study, an extensive survey on false smut of rice was conducted during Kharif 2010 and 2011 in rice grown fields of districts Kulgam, Anantnag, Pulwama, Budgam and Srinagar of Kashmir division which represent the temperate area of the country for rice cultivation. Random samples of 50-100 hills were selected at the time of harvesting. Disease assessments were carried out according to Singh and Dube (1978). Number of infected tillers and infected grains in panicles were recorded and the disease incidence (No. of smut balls /plot) and disease severity (No. of smut balls /infected panicle) was calculated. Further, to study the effect this disease on seed germination and seedling traits some smutted and non-smutted panicles collected during survey were threshed separately and seeds were grown on moistened germination sheet in Petri dishes under *in vitro* conditions using seed germinator for 5 days at 24±2^{0}C. Data were recorded on seed germination (%), shoot length (cm) and root length (cm) and per cent reduction in these traits over control were worked out. Besides, an additional experiment was conducted to know the reaction of some elite fine grained aromatic rice genotypes to false smut in field under temperate agro-climatic conditions of Kashmir. Since viable and proven cultures of *U. virens* known to be virulent to rice were not available, spores of the fungus were collected from pseudomorphs found on panicles of field-grown 'Pusa sughandh 3' harvested in 2009-2010. Panicles and seeds containing the pseudomorphs had been stored in the laboratory at 24°C until used. Spores were suspended in water and used without additional surfactants added to the spore suspensions. Spores were diluted to a concentration of 1 million spores per ml. Fifty gram seeds of eight advanced fine grain aromatic genotypes of rice and two commercial varieties (Table 3) were placed in 20 ml of inoculum. Inoculation was accomplished by infiltration of seeds for 30 min with a protocol adapted from Zhou et al. (2008) for 30 min. After infiltration, seeds were air-dried overnight at 24°C. Seeds were germinated in Petri dishes on moistened filter paper for 72 hours before direct transplantation to the plastic pots. Further, thirty days old seedlings of all the eight genotypes of rice including two commercial varieties were transplanted during June 2010 and 2011 at Mountain Research Centre for Field Crops, Khudwani, Kashmir at an altitude of 1560m amsl (above mean sea level). The seedlings were transplanted in five rows of 5m length with inter and intra-row spacing of 20 x 15 cm, respectively in a Randomize Complete Block Design with three replications. The recommended packages of practices were followed to raise ideal crop stand except plant protection measures for false smut disease. Panicles were harvested as they matured for each of the replication from different cultivars used. Data were recorded for number of panicles infected, number of smutted grains per panicle, per cent smutted grains and disease incidence and severity were recorded for each genotype as discussed earlier. Further the genotypes were also evaluated for various economic traits like plant height (cm), days to flowering and maturity, panicle per square meter and grain yield (q/ha). Data were statistically analyzed using CPCS-1 software package developed by Cheema and Singh (1993) following analysis of variance (Gomez and Gomez 1984) Critical difference was worked out to compare the mean values and inferences were drawn accordingly.

Results and discussion

Rice false smut is a new threat to the rice production under Kashmir agro-climatic conditions. This is the first record of false smut of rice in Kashmir. The infected grains were transformed into large velvety, green masses. Initially the chlamydospores were orange yellow which later turned to dark green or almost black (Fig.1).

Fig.-1: False smut of rice showing typical symptoms of dark green and black

False smut of rice is reported in most rice growing areas of the world (Mehrotra 1990) and its typical symptom were earlier described by Verma and Singh (1988). In this study, an extensive survey on false smut of rice was conducted at the time of maturity and harvesting in rice grown fields of districts Kulgam, Anantnag, Pulwama, Budgam and Srinagar of Kashmir division during Kharif 2010 and 2011 which represent the temperate area of the rice cultivation. The disease was more or less prevalent in all the locations surveyed. The disease incidence and intensity varied with respect to location as well as season (Table 1). The disease incidence in 2010 ranged from 6.92 (Pulwama) to 18.94 (Budgam) per cent while it was significantly higher ranging from 10.47 (Pulwama) to 25.46 (Budgam) per cent with an average of 15.83 % in 2011. The yearly averages reflected that amongst the various districts surveyed, Budgam recorded highest (22.2%) disease incidence followed by Kulgam (13.2%). The corresponding values for disease intensity for both the districts were 4.10% and 2.87%, respectively. The seasonal and location specific differences regarding the incidence of false smut of rice has also been documented in previous studies (Li et al. 1986; Biswas 1999). They reported 27 % rice false smut incidence in India, while in China it ranged from 1-10% until 50-60% in years of severe disease.

Table-1: Disease status of rice false smut in different districts of Kashmir

S. No.	District	Disease incidence (%)			Disease intensity (%)		
		2010	2011	Mean	2010	2011	Mean
1	Kulgam	11.50	15.06	13.28	2. 02	2.87	2.87
2	Anantnag	8.47	12.35	10.41	1.50	2.30	1.90
3	Pulwama	6.92	10.47	8.69	0.58	1.39	0.98
4	Budgam	18.94	25.46	22.2	3.42	4.79	4.10
Mean		11.45	15.83	-	1.83	2.83	-
C.D. (0.05) for location: 2.45; years: 3.20; location x year: 3.85					location: 1.35; years: 0.60; location x year: 2.05		

Figure 2 shows the difference in seed germination for the samples taken from smutted and non-smutted panicles collected during survey from the rice variety 'Pusa sugandh-3'. Results appended in Table 2 revealed that false smut of rice had significant effect of on seed germination and seedling traits. Seed germination in smutted samples ranged from 65.0 to 77.0 per cent with an average of 72.4% which is 21.45 per cent lower than the non - smutted samples (92.20%).

Table 2 Effect of false smut of rice on seed germination and seedling growth under *in vitro* conditions

Traits/ S. No	Seed germination (%)		Shoot length (cm)		Root length (cm)	
	Smutted panicles	Non-smutted panicles	Smutted panicles	Non-smutted panicles	Smutted panicles	Non-smutted panicles
1	77.0	95.7	3.5	4.7	5.2	6.0
2	75.6	93.7	2.8	4.8	4.4	6.5
3	72.0	90.4	3.2	5.1	4.8	6.7
4	65.0	88.6	3.4	4.5	5.4	6.4
Mean ± SE	72.4 ± 3.25	92.2 ± 1.80	3.22 ± 0.35	4.78 ± 0.40	4.95 ± 0.6	6.40 ± 0.15
Per cent reduction	21.45	-	32.53	-	22.65	-

Similarly, shoot length and rood length were also affected by this disease and reduced to the extent of 32.53 and 22.65 per cent, respectively in comparison to non- smutted. Reduction in seed germination and seedling traits in rice due to infection by spores of *Ustilaginoidea virens* has also been reported by Schroud and TeBeest (2005). The comparison of seedlings from smutted and non smutted samples is presented in Fig. 3. This experiment shows that infection of rice plants can occur through planting of infested seeds and those plants grown from infested seed encounter a reduction of seed germination and seedling growth.

Fig 2.Effect of false smut disease on seed germination in rice

Further, eight advanced fine grain aromatic genotypes of rice and two commercial varieties were tested for their susceptibility to false smut after natural infections under field conditions during Kharif seasons 2010-2011. Disease assessment calculated at the harvesting time showed that rice genotypes differ in the disease incidence, which appeared at different degree of infection with rice false smut. Amongst the genotypes tested mean disease incidence and severity over seasons varied from 2.10-34.11 per cent and 0.55-14.47 per cent, respectively (Table 3).

Table-3: Reaction of rice cultivars to false smut at Mountain Research Station for Field Crops, Khudwani during 2010-2011

S. No.	Genotypes	Disease incidence (%)			Disease intensity (%)		
		2010	2011	Mean	2010	2011	Mean
1	SKUA-417	1.3	2.9	2.10	0.2	0.9	0.55
2	Pusa Sungadh -5	25.63	28.25	26.94	12.50	13.87	13.18
3	SKUA-419	5.87	8.46	7.16	2.40	4.50	3.45
4	SKUA-420	18.50	22.30	20.40	9.34	10.90	10.12
5	SKUA-421	9.45	14.76	12.10	4.63	5.67	5.15
6	SKUA-422	12.00	17.54	14.77	6.89	8.03	7.46
7	SKUA-423	22.42	25.21	23.81	10.04	12.00	11.02
8	Pusa Sungadh-3	31.67	36.55	34.11	13.47	15.48	14.47
9	Jhelum	0.00	0.00	0.00	0.00	0.00	0.00
10	Shalimar Rice-I	0.00	0.00	0.00	0.00	0.00	0.00
Mean		15.85	19.49		7.43	8.91	
C.D. (0.05) for genotypes: 3.65; years: 2.12; genotype x year: 4.65					genotypes: 1.65; years:0.68; genotype x year: 2.82		

Singh et al. (1987) and Biswas (2001) have reported that rice cultivars played an important role in the degree of rice false smut infection. In this study, two commercial cultivars of rice, Jhelum and Shalimar Rice-1 were found resistant to false smut of rice. These cultivars are short duration and took 137 and 142 days to maturity, respectively and are have medium bold grain type (Table 4). Among the aromatic fine grained genotypes the minimum per cent incidence of disease and severity were recorded for genotype SKUA- 417 (2.10, 0.55) followed by SKUA-419 (7.16, 3.45) showing moderate level of resistance. However, cultivar Pusa Sungadh-3 showed the highest disease incidence and intensity (34.11 and 14.47 respectively) followed by Pusa Sungadh-5 (26.94 and 13.18 respectively) which took 153.20 and 157.60 days to maturity, respectively during the two successive growing seasons (Table 4).

Table-4 : Mean economic characters of rice genotypes studied during 2010 and 2011

S. No.	Designation	Plant height (cm)	Days to 50% flowering	Days to maturity	Panicle density/m^2	Grain type	Grain yield (q/ha)
1	SKUA-417	84.33*	108.00	149.60	403.00	LS	70.28
2	Pusa Sungadh -5	94.80	114.00	157.60	247.00	LS	52.80
3	SKUA-419	81.00	108.00	149.30	353.00	LS	77.65
4	SKUA-420	81.40	107.00	149.60	343.00	LS	73.21
5	SKUA-421	84.60	106.00	149.30	356.00	LS	60.69
6	SKUA-422	92.20	109.00	155.30	396.00	LS	69.01
7	SKUA-423	80.10	109.00	145.60	396.00	LS	64.54
8	Pusa Sungadh-3	89.40	120.00	153.20	326.00	LS	61.00
9	Jhelum	106.80	96.00	137.60	363.00	MB	73.70
10	Shalimar Rice-I	121.90	106.00	142.30	349.00	MB	82.58
C.D. (0.05)		6.20	4.32	5.85	85.25	-	5.76

LS- Long slender; MB-Medium bold

Differential response of rice genotypes to false smut were also reported (Sugha et al. 1992; Biswas 2001; Atia 2004; Kurauchi et al. 2006). The similar efforts have been made to identify and utilize rice germplasm with false smut resistance. Some resistant lines have been identified but account for a tiny proportion of the rice germplasm. It was observed that early maturing genotypes were escaped from the disease. These results are in complete agreement with reports of Singh and Khan (1989). The differences between tested rice cultivars to false smut might be attributed to differences in genetic makeup of tested cultivars in addition to environmental factors that might affect the host-pathogen interaction (Walker 1975).

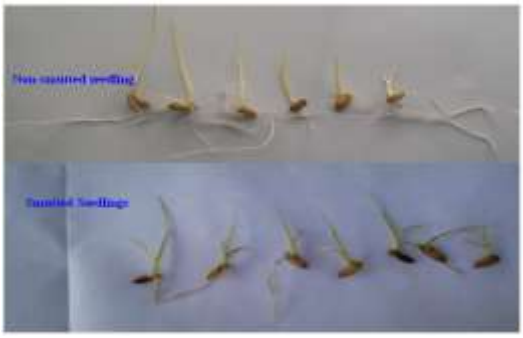

Fig.3 Seedling growth from smut infected and non- smutted grains samples

Kashmir valley being mono cropped area of rice in Kharif season, further studies are needed to investigate the viability of the fungal spores and sclerotia during winter, variation between fungal isolates, ability to produce mycotoxins and devise control measures using integrated disease management practices. In long term strategy this new challenge in future can be tackled by successful breeding programme by using resistant lines identified in the study as donors and most adapted but susceptible varieties as recurrent parents and to bred varieties which are resistant/tolerant to the disease.

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